REMARKS

Applicant appreciates the courtesy extended by Primary Examiner Jeffrey S. Parkin, during two separate telephone interviews with Applicant's representatives, Xu Zhang and Cecilia Lopez-Chua, on December 20, 2005 and April 28, 2006, respectively.

During the first interview conducted with Xu Zhang, Examiner Parkin expressed concerns over the relative nature of the salt and protein conditions used in the capsid assembly triggering process and suggested that Applicant "provides further clarification concerning the various salt concentrations in the various solutions employed to avoid ambiguity or confusion.

In the second interview, Examiner Parkin advised Applicant's representative, Cecilia Lopez-Chua, to file a Request for Continued Examination (RCE) application to include an amended claim 1 and a new claim 25. In addition, Examiner Parkin proposed that Applicant's representative should contact the Examiner after one month of the RCE filing to discuss the application further.

Claims 1, 4, 7-8 and 11-12 remain pending. New claim 25 has been added. In addition, claims 2-3, 5-6, 9-10, and 13-18 are cancelled and claim 19-24 are withdrawn, from consideration. Applicant requests rejoinder of the withdrawn claims upon allowance of the pending claims.

The amendments to the claims and addition of the new claim are supported by the entire specification, particularly at Example 2 of the specification. Applicant respectfully submits that the above amendments do not introduce new matter. Accordingly, Applicant respectfully requests the Examiner to enter these amendments.

Claim Objection

The Examiner objected to the recitation of the abbreviated term of human immunodeficiency virus (HIV) in claims 1, 4, 7-8 and 11-12. To overcome this objection, Applicant has appropriately amended the claim language in claim 1 to properly define this term.

Based on the foregoing, Applicant respectfully submits that this objection is rendered moot. Accordingly, reconsideration and withdrawal of this objection is, respectfully requested.

Rejection Under 35 U.S.C. § 112 (Second Paragraph)

At pages 1-2 of the Office Action, the Examiner rejected claims 1, 4, 7-8 and 11-12 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant respectfully traverses the rejection.

In rejecting the above-mentioned claims, the Examiner asserted that the viral assembly triggering process of "rapidly increasing the salt concentration" is vague and indefinite to a skilled person. In addition, the Examiner contends that the phrase "rapidly increasing the salt concentration" is "relative." Consequently, the Examiner has required the Applicant to explain certain parameters used in the assay, *e.g.*, what constitutes a rapid increase in salt concentration and what salts are to be employed in the assay. Besides these parameters, the Examiner, during the first telephonic interview with the Applicant's representative, Xu Zhang, expressed concerns over two additional issues, namely, the concentrations of added salt that would induce self-assembly and concentrations of the capsid protein that would result in self-assembly (see page 3 of the Office Action Summary mailed November 29, 2005).

At the outset, method claim 1, as amended, now recites a capsid assembly triggering process that is performed by rapidly increasing the salt concentration in the HIV capsid protein solution such that when capsid assembly occurs, the final concentration of the salt concentration is at least 1 M sodium salt. As illustrated in Example 2 and Figure 4 of the specification, the capsid assembly triggering process can be detected within 20 seconds after salt increase. In addition, the specification, at pages 18-24, teaches that the capsid assembly can be triggered at a salt concentration range that ranges from 45 μM – 2.25M sodium salt and at a capsid protein concentration that ranges from 38 – 350 μM.

Accordingly, a skilled person, upon reading the specification and claims, would understand what comprises a rapid increase of salt concentration, what salts are to be employed in the assay, and the concentration of added salt and capsid protein that would induce and result in self-assembly.

In light of the aforementioned remarks and claim amendments, Applicant respectfully submits that claim 1, as well as its dependent claims, is clear and definite. Reconsideration

and withdrawal of the rejection based on section 112, second, paragraph, is earnestly requested.

CONCLUSION

For at least the reasons set forth above, Applicant respectfully submits that this application is in condition for allowance. Favorable consideration and prompt allowance of the claims are earnestly requested. A fee for a three (3)-month extension of time is due for filing this response. The Commissioner is hereby authorized to charge any payment deficiency to Deposit Account No. 19-2380 referring to attorney docket number 057909-011000.

Should the Examiner believe that anything further is desired in order to place the application in even better condition for allowance, the Examiner is invited to contact Applicant's undersigned representative at the telephone number listed below.

Respectfully submitted,

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EXAMPLE 2

Assembly of HIV-1 Capsid Protein

The HIV-1 capsid (CA) protein plays a crucial role in both assembly and maturation of the virion. The present invention develops a method for monitoring capsid assembly to characterize capsid-capsid interactions involved in capsid assembly. Monitoring the process of polymer assembly with kinetic analysis has been well established for many years. The *in vitro* assembly of the Tobaco Mosaic virus was the first to be studied with kinetic analysis. Similar techniques have also been applied to the assembly of P22 in order to elucidate key steps in the assembly process. Analyzing the kinetics of capsid assembly provided a sensitive method for monitoring the effects of capsid mutations and inhibitory compounds on capsid-capsid protein interactions involved in assembly.

A dilution technique was developed to trigger capsid assembly instead of dialysis into 1 M NaCl in order to rapidly transfer the assembly reaction into the ultraviolet (UV) light spectrometer cuvettes for analysis. The proteins were dialyzed into 50 mM Na_2HPO_4 at pH 8.0 and the capsid protein was concentrated

to 1 mM. The capsid protein was then diluted with 50 mM Na_2HPO_4 at pH 8.0 to give the final concentration necessary for the assembly reaction at a volume of 197 uL. The capsid protein was triggered to polymerize by the addition of 197 uL of 50 mM Na_2HPO_4 4 M NaCl at pH 8.0 to produce a final 2.25 M NaCl concentration. Initial assembly reactions were done using a final 1 M NaCl concentration. Following the addition of NaCl the assembly reaction was rapidly mixed and place into a cuvette.

Approximately 20 sec elapsed between when capsid protein was triggered to assemble and the first time point was monitored. The assembly of capsid protein into larger polymers was followed as an increase in optical density. The large polymers formed during capsid assembly caused an increase in light scattering that was observed as an increase in the solutions turbidity (optical density). The increase in optical density was monitored on a spectrometer at 350 nm every 20 sec for 0.5sec. The data points representing the largest increase in optical density were linear fit using the origin fitting program to obtain the slope of the line tangent to these data points. For the assembly reactions with either the N-domain or C-domain present they were mixed with the full-length capsid protein prior to triggering assembly.

The present invention provides a method to monitor the kinetics of *in vitro* capsid protein (CA) assembly. In these studies HIV-1 capsid protein was assembled by diluting in concentrated NaCl to produce a final concentration of 1 M NaCl instead of dialyzing capsid protein into 1 M NaCl, thus allowing rapid transfer of the assembly reaction to the cuvette for analysis. The capsid protein assembly rate was dependent on capsid concentration and the capsid protein C-domain interactions. Analyzing the kinetics of capsid assembly provides a sensitive method for monitoring and screening mutations and inhibitory compounds that modulate capsid-capsid protein interactions involved in assembly.

The present invention also provides a novel mass spectrometry based approach to measure hydrogen/deuterium exchange profiles is used to identify intersubunit interfaces and characterize domain stability. This is complemented with Raman spectroscopy-based studies of subunit structure and stability. Selected wild type and mutant proteins which have been shown to polymerize into physiologically relevant forms are characterized and then use this information to characterize mature and immature enveloped virus-like particles budded from cells grown in culture. These studies provide otherwise unobtainable information on the